

The University of Chicago

UTILIZATION OF ORGANIC AND
INORGANIC IRON BY THE
NORMAL INFANT

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF HOME ECONOMICS
AND HOUSEHOLD ADMINISTRATION

1939

By

HELEN OLDHAM

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
AMERICAN JOURNAL OF DISEASES OF CHILDREN
Vol. LIV, No. 2, August 1937

The University of Chicago

UTILIZATION OF ORGANIC AND
INORGANIC IRON BY THE
NORMAL INFANT

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF HOME ECONOMICS
AND HOUSEHOLD ADMINISTRATION

1939

By
HELEN OLDHAM

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
AMERICAN JOURNAL OF DISEASES OF CHILDREN
Vol. LIV, No. 2, August 1937



UTILIZATION OF ORGANIC AND INORGANIC IRON BY THE NORMAL INFANT

In this study the relative availability of inorganic and of organic iron in the diet of the normal infant has been investigated by means of a study of the iron balances over a long period. Interest in the problem was stimulated by the reports of Elvehjem and his colleagues,¹ which indicated that in the case of rats only the inorganic portion of the dietary iron was utilized.

Until now practically all investigators interested in determining the "availability" of organic and inorganic or ionizable iron for hemoglobin synthesis have used anemic rats for their feeding experiments. Elvehjem and his co-workers¹ found consistently that in the case of the anemic rat only the inorganic portion of the iron in the diet is utilized for the formation of hemoglobin. They found this to be true when a number of foods were given and the hemoglobin response was compared with that manifested when amounts of ferric chloride equivalent in some experiments to the total iron, and in others to the inorganic iron, of the food were given. Since organic iron occurs in animal and in plant foods in the form of hematin or hematin-like compounds, Elvehjem² also fed iron in the form of hematin. The hemoglobin showed no gain, and analyses of the livers of the experimental animals showed that no iron had been stored in them. This indicated the inability of the

Submitted by Miss Oldham in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

From the Departments of Pediatrics and Home Economics of the University of Chicago. The study was supported in part by a grant from the Douglas Smith Foundation for Medical Research of the university.

1. (a) Elvehjem, C. A.; Hart, E. B., and Sherman, W. C.: The Availability of Iron from Different Sources for Hemoglobin Formation, *J. Biol. Chem.* **103**:61 (Nov.) 1933. (b) Sherman, W. C.; Elvehjem, C. A., and Hart, E. B.: Further Studies on the Availability of Iron in Biological Materials, *ibid.* **107**:383 (Nov.) 1934. (c) Elvehjem, C. A.; Hart, E. B., and Sherman, W. C.: The Limitations of Cereal Milk Diets for Hemoglobin Formation, *J. Pediat.* **4**:65 (Jan.) 1934.

2. Elvehjem, C. A.: The Relative Value of Inorganic and Organic Iron in Hemoglobin Formation, *J. A. M. A.* **98**:1047 (March 26) 1932.

rat to assimilate the iron of the hematin molecule. From findings in the various studies to which reference has been made, Elvehjem and his associates concluded that only the inorganic portion of the iron of food was utilized.

Vahlteich, Rose and MacLeod,³ however, did not agree with that conclusion. When they fed whole wheat (which is reported by Elvehjem to have only 47 per cent of its iron in the inorganic form) to anemic rats, they found a hemoglobin response comparable to that when they fed ferric chloride equivalent in amount to the total iron in the whole wheat, the copper content of the diets being constant. When the wheat was predigested before it was used, they found a higher hemoglobin response than with ferric chloride. They considered digestibility to be a factor in very young and very anemic animals.

Widdowson and McCance⁴ calculated the total intake of iron and the intake of inorganic iron of a number of adults whose diets were weighed. In one group the inorganic iron constituted more than 90 per cent of the total, and in the other, less than 50 per cent. The investigators could find no significant correlation between the hemoglobin levels and either the total intakes of iron or the intake of inorganic iron. They expressed the opinion that although the intake of iron may be a factor in regulating the hemoglobin level, other factors predominated under the conditions of their study.

The fact that animals under some conditions appeared to be unable to utilize the organic portion of the iron in their food made the question as to whether children also would be unable to utilize that portion seem worthy of investigation. The present study was begun with this idea in mind. It was felt also that a study of this type would furnish some information concerning the iron requirement of the infant, about which little was known.

EXPERIMENTAL PROCEDURE

The subject of this experiment was a normal, healthy infant. The study of his iron metabolism was not started until he was 6 months of age, at which time it was felt that any iron stores present at birth would have been largely depleted. During the eight months of the study, there were but three interruptions. One diet proved to be constipating, and it was changed; the other two interruptions were due to slight infections of the upper respiratory tract. Each time the study was discontinued until the infant had entirely recovered. His daily life was as nearly normal as possible, and as a result he developed much the same way as would any normal child. He sat without assistance at 8 months of age and stood alone when he was 1 year old. His weight and height during the entire

3. Vahlteich, E. M.; Rose, M. S., and MacLeod, G.: Effect of Digestibility upon the Availability of Iron in Whole Wheat, *J. Nutrition* **11**:31 (Jan.) 1936.

4. Widdowson, E. M., and McCance, R. A.: Iron in Human Nutrition, *J. Hyg.* **36**:13 (March) 1936.

study were in the high normal range according to the Woodbury⁵ standards from the Children's Bureau of the United States Department of Labor, 1921.

Foods were selected which for the most part are commonly used in infant feeding, and therefore the diet was comparable to that of a normal child of the same age. Five mixed formula feedings were given daily until the infant was 10 months of age, at which time he was put on four formula feedings a day. At this time feeding with a spoon was started, a cereal which contained small amounts of iron being used.⁶ Gradually, as the child became accustomed to the spoon, and it was felt that feedings given with it could be as accurately measured as could bottle feedings, the major portion of his solid foods was given with a spoon. At the age of 1 year he was receiving three meals a day.

The infant took his food exceptionally well and seldom refused any. The formula bottles were rinsed with distilled water and the rinsings either given after the feeding or added to the next feeding to insure no loss. A weighed piece of gauze was placed about the child's neck at each feeding time, and any regurgitations were calculated from the gain in the weight of the gauze during the feeding. The total weight of the regurgitations was in every case much less than the experimental error incurred in the analyses of the food. Any small amounts of food which were refused were analyzed for iron.

The iron metabolism was studied for twenty-eight periods of five days each. During the first twelve periods a metabolism frame was used, and in that portion of the study the same diet was given for a period of five days when no collections were made and then for three successive five day collection periods, with intervals between them of two days when no collections were made.

When the child had reached the age of 10½ months a nursery chair with a specially constructed harness was used for the collection of feces. As a result of this, collections could be made continuously, although the stools were marked off with carmine for each five day period as had been done during previous periods. The infant was regular in his elimination, and little difficulty was encountered in training him. No collection of urine was made during the last sixteen periods, but as the amount of iron in the urine is always less than 1 per cent of the total excretion of iron, it seemed justifiable to consider it constant and make allowance for it in calculating the total excretion. Furthermore, the child was at the age when it was necessary that he be allowed to exercise freely for proper development. When the nursery chair rather than a metabolism frame was used, he could spend the greater part of the day in a play pen on the sun porch or in the play room in the company of other children. It was felt that his development under these conditions would closely approach that of any normal child.

The diets for each period as well as the amount of each food given daily are shown in table 1. As many foods as possible were obtained in quantities sufficient to last for several months to insure uniformity in quality and consequent constancy of the intake of iron. Food for each day was weighed on a platform balance and was transferred quantitatively to the bottles used for feeding the formula. Powdered milk,⁷ cane sugar, orange juice, cod liver oil and a cereal were used

5. Woodbury, R. M.: *Statures and Weights of Children Under Six Years of Age*, Bulletin 87, U. S. Dept. of Labor, Children's Bureau.

6. Cream of Wheat was the cereal used.

7. Powdered milk was furnished, in the form of Dryco, by the Dry Milk Company.

in the diet throughout the entire study. A strained vegetable⁸ also was included during all but the four periods when beef and cellulose were fed.

The general plan of the study was to note the retention of iron and the formation of hemoglobin at several levels of intake and with different proportions of inorganic and organic iron at each level. That is, a given amount of iron, with as low a percentage of iron in the inorganic form as possible, was fed for at least three periods; the same amount of iron, with a high percentage in the inorganic form, was fed for an equal number of periods. The same procedure was then

TABLE 1.—*Dietary for the Entire Study*

Period No.	Diet	Calories	Copper Content, Mg. per Day
1-3	Basic diet 1*..... 20 Gm. Cream of Wheat..... 70 Gm. strained spinach.....	860	0.22
4-6	Basic diet 1..... 20 Gm. Cream of Wheat..... 50 Gm. strained peas.....	860	0.26
7-9	Basic diet 1..... 20 Gm. special Baby Ralston..... 240 Gm. strained spinach.....	875	0.30
10-12	Basic diet 1..... 20 Gm. special Baby Ralston..... 100 Gm. strained peas.....	875	0.36
13-16	Basic diet 1..... 30 Gm. Cream of Wheat..... 100 Gm. strained peas..... Soluble ferric pyrophosphate = 1.68 mg. iron..... Cupric sulfate = 0.09 mg. copper.....	915	0.51
17-20	Basic diet 1..... 30 Gm. Cream of Wheat..... 100 Gm. strained peas..... 3 Gm. VioBin.....	925	0.49
21-22	Basic diet 1..... 30 Gm. Cream of Wheat..... 50 Gm. strained peas.....	900	0.28
23-26	Basic diet 2†..... 30 Gm. Cream of Wheat..... 100 Gm. beef..... 10 Gm. cellu-flour.....	920	0.34
27-28	Basic diet 1..... 40 Gm. Cream of Wheat..... 150 Gm. strained peas..... Soluble ferric pyrophosphate = 0.89 mg. iron.....	970	0.55

* Basic diet 1 consisted of 115 Gm. powdered milk, 45 Gm. cane sugar, 60 cc. orange juice and 8 cc. cod liver oil.

† Basic diet 2 was the same as basic diet 1 except that the amount of powdered milk was reduced to 100 Gm.

repeated with a larger amount of iron. Only such foods were used as are generally considered suitable for the diet of other children of the same age, and the amounts fed were not above those which are given normally.

In the first six periods the total daily intake of iron was kept constant at 2.6 mg. The daily intake of inorganic iron during periods 1 to 3 was 1.9 mg. but was raised to 2.2 mg. during periods 4 to 6. This difference in the amount of inorganic iron was the result of feeding spinach in the first three periods and peas in the last three. The same cereal was given throughout the entire six periods.

8. All vegetables were supplied by the Gerber Products Company.

During periods 7 to 12 the total daily intake of iron was 3.6 mg. The daily intake of inorganic iron was 2.7 mg. in periods 7 to 9 and 3.4 mg. in periods 10 to 12. The change in the amount of inorganic iron was again the result of substituting peas for spinach. A different cereal⁹ with a higher iron content was used throughout periods 7 to 12 which, with the increased amounts of vegetables, accounted for the higher level of the intake of iron.

The total daily intake of iron in periods 13 to 20 was 4.8 mg., of which 4.1 to 4.6 mg. was inorganic. The infant received peas and cereal during the entire time but in periods 13 to 16 received additional iron as soluble ferric pyrophosphate and copper as cupric sulfate. In periods 17 to 20 he was given 3 Gm. daily of a commercial wheat germ product¹⁰ high in vitamin B, which contained the same amounts of iron and copper given as salts in the four previous periods.

In periods 21 and 22 the total daily intake of iron was reduced to 2.7 mg. by using a cereal of lower iron content and a smaller amount of peas. These periods on a lower level of intake were introduced to deplete any stores which might have been built up as a result of the higher intake of the previous periods.

After feeding this lower level for two periods, cooked ground beef was gradually introduced into the diet, until after a few days the infant was receiving 100 Gm. daily. The beef was purchased as round steak, all the fat was removed and the lean was finely ground in a meat grinder. Sufficient lean beef for the daily feeding was packed into a small pyrex beaker, the top of which was covered tightly with waxed paper. The meat was then cooked thirty minutes under steam pressure. A sample for analysis was treated similarly. During periods 23 to 26 the child received beef and a purified cellulose flour¹¹ but no vegetable. The amount of powdered milk received during these periods was slightly less than in previous periods because of the additional calories and protein in the meat. The total daily intake of iron during periods 23 to 26 was 5.2 mg., only 3.2 mg. of which was inorganic iron.

The total daily intake of iron in the last two periods was kept at 5.3 mg., but the intake of inorganic iron was raised to 4.6 mg. by the substitution of peas and soluble ferric pyrophosphate for the beef and cellulose flour. The amount of powdered milk was again raised to the level fed previous to period 23.

The total amount of iron in food and excreta was determined by a modification of Stugart's method,¹² which we¹³ have described elsewhere. The urine was collected in glass bottles, and it was preserved with thymol and by refrigeration until the end of each five day period, when it was thoroughly mixed and measured.

9. A special form of Baby Ralston, without the added iron and calcium salts, was furnished by the Ralston Purina Company.

10. VioBin, a stabilized, defatted product of the germ of wheat, was furnished by the VioBin Corporation.

11. The cellulose flour was purchased in the form of Cellu-Flour. Since it contained appreciable amounts of iron, the iron was removed by treating with hot hydrochloric acid. After the mixture had been filtered, the flour was washed with distilled water until the washings showed no reaction to the test for chlorides. The purified product was then analyzed for iron, and the amount received in the daily ration was added to the intake of iron in other forms.

12. Stugart, R.: Determination of Iron in Milk and Other Biological Materials, *Indust. & Engin. Chem. (analyt. ed.)* **3**:390 (Oct.) 1931.

13. Schlutz, F. W.; Morse, M., and Oldham, H.: The Influence of Fruit and Vegetable Feeding upon the Iron Metabolism of the Infant, *J. Pediat.* **3**:225 (July) 1933.

Feces were collected in porcelain dishes, and the total amount for the period was dried to constant weight and ground in a porcelain mortar. Aliquots of each food were taken every day and made into separate composites which were analyzed at the end of each period. In the case of the powdered milk, cane sugar and cereals, which were obtained in quantities, the contents of each lot were thoroughly mixed, sampled and analyzed, making it unnecessary to take daily aliquots. Care was taken to keep food from being contaminated with extraneous iron after aliquots for analyses had been taken. Distilled water was used in making up the formulas. Pyrex glassware exclusively was used for cooking purposes and analyses. Ashing was done in platinum dishes.

The amount of inorganic iron was determined by various modifications of Hill's¹⁴ *a, a'*-dipyridine method. The earlier modifications of this method made by the Wisconsin group¹⁵ and those made by Shackleton and McCance¹⁶ gave similar results. Since a supply of purified sodium hydrosulfite was available, it was used as a reducing agent instead of hydroquinone. Results of analyses were more consistent when blanks were used to counteract the effect of plant pigments than when the pigments were precipitated with lead acetate before making the colorimetric comparison according to later modifications of the *a, a'*-dipyridine method made by Kohler and his associates.¹⁷ In precipitating the plant pigments with lead acetate the resulting solution was often cloudy even after centrifugating and filtering, and a colorimetric comparison was impossible. Because of this difficulty colors were compared with those of a set of standards by using a comparator.

When changes in diet were made, a composite of foods which represented a portion of an average day's intake was weighed out, and the amount of copper in the mixed sample was determined by Daniels and Wright's¹⁸ modification of McFarlane's¹⁹ method. No attempt was made to analyze the excreta to determine the amount of copper present.

The amount of hemoglobin was determined weekly by the Newcomer method.

Nitrogen balances were ascertained every third period during the first twelve periods. The child showed a strongly positive nitrogen balance during these periods. As he was gaining in weight regularly, it was felt that to determine the nitrogen balances in the later periods would be of no significant value in this study.

COMMENT ON RESULTS

Both the total intake of iron and the intake of inorganic iron as well as the corresponding retention and the values of hemoglobin for the twenty-eight periods are shown in the chart. Results of the analyses of food and excreta are listed in tables 2 and 3.

14. Hill, R.: A Method for the Estimation of Iron in Biological Material, *Proc. Roy. Soc., London*, s.B **107**:205 (Nov.) 1930.

15. Elvehjem, Hart and Sherman.^{1a} Sherman, Elvehjem and Hart.^{1b}

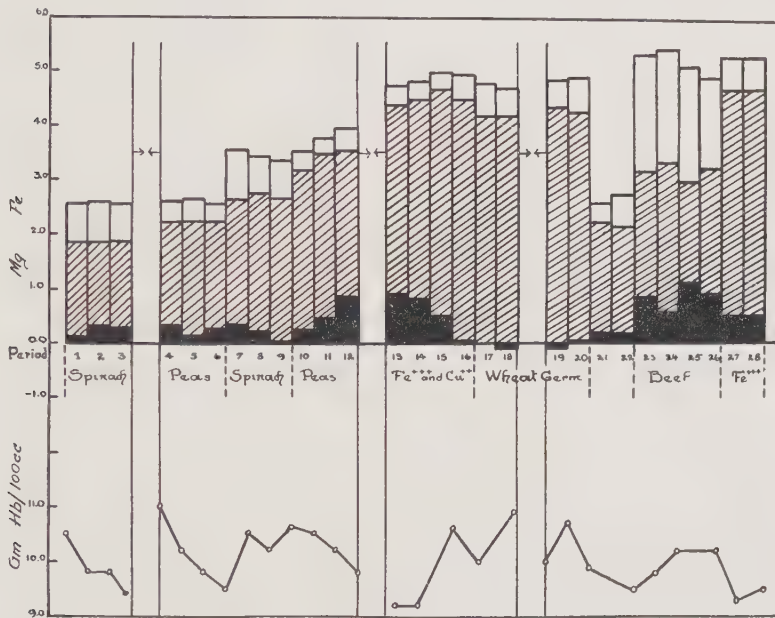
16. Shackleton, L., and McCance, R. A.: The Ionisable Iron in Foods, *Biochem. J.* **30**:582 (April) 1936.

17. Kohler, G. O.; Elvehjem, C. A., and Hart, E. B.: Modifications of the Bipyridine Method for Available Iron, *J. Biol. Chem.* **113**:49 (Feb.) 1936.

18. Wright, O. E.: Personal communication.

19. McFarlane, W. D.: Application of the Sodium Diethyldithiocarbamate Reaction to the Micro-Colorimetric Determination of Copper in Organic Substances, *Biochem. J.* **26**:1022 (June) 1932.

The irregularity of the retentions as measured in the first twelve periods made it difficult to determine the availability of the organic iron of vegetables. The results are therefore somewhat ambiguous. In the first six periods, with the total daily intake of iron at 2.6 mg., it made no difference whether the intake of inorganic iron was 1.9 mg., as in periods 1 to 3, or 2.2 mg., as in periods 4 to 6. The average retentions were 0.24 mg. for the first three periods and 0.25 mg. for the next three periods, and they paralleled the total intake of iron rather than the intake of inorganic iron. On the other hand, in periods 7 to 12, with a higher total intake of iron (3.6 mg.), the retention was higher



The total intake of iron for each period is represented by the entire column; the intake of inorganic iron, by the cross-hatched portion of the column, and the retention of iron, by the black portion. The values of hemoglobin also are shown. The arrows indicate the intervals during which the study was interrupted.

in the last three periods, coincidentally with a higher intake of inorganic iron.

Whether this higher retention was actually related to the higher intake of inorganic iron, or to the higher total intake of iron which took place during the last two periods on account of the higher iron content of the peas and the orange juice given at that time, is open to question. The fact that the retention in period 10 was comparable to those in preceding periods, although the intake of inorganic iron was decidedly

higher, again made it seem that the percentage of inorganic iron in the intake does not influence the retention. When represented graphically, either as total iron or on a milligram per kilogram basis, the trend of the retention seemed to follow that of the total intake of iron more

TABLE 2.—*Total Intake of Iron, Intake of Inorganic Iron, Total Output of Iron and Corresponding Retention*

Period No.	Total Intake of Iron, Mg. per Day	Intake of Inorganic Iron, Mg. per Day	Intake of Inorganic Iron, Percentage of Total Intake	Total Output of Iron, Mg. per Day	Retention of Iron, Mg. per Day
1	2.58	1.85	72	2.45	+0.13
2	2.60	1.84	71	2.29	+0.31
3	2.56	1.87	73	2.27	+0.29
4	2.61	2.21	85	2.28	+0.33
5	2.66	2.22	83	2.52	+0.14
6	2.52	2.22	88	2.25	+0.27
7	3.55	2.62	74	3.22	+0.33
8	3.41	2.74	80	3.20	+0.21
9	3.35	2.63	78	3.33	+0.02
10	3.53	3.18	90	3.29	+0.24
11	3.79	3.48	92	3.32	+0.47
12	3.98	3.53	89	3.14	+0.84
13	4.71	4.38	93	3.81	+0.90
14	4.80	4.45	93	3.99	+0.81
15	4.97	4.66	94	4.46	+0.51
16	4.90	4.44	91	4.85	+0.05
17	4.76	4.16	87	4.75	+0.01
18	4.68	4.18	89	4.79	-0.11
19	4.81	4.32	90	4.91	-0.10
20	4.89	4.22	86	4.82	+0.07
21	2.59	2.22	86	2.38	+0.21
22	2.75	2.15	78	2.57	+0.18
23	5.29	3.17	60	4.44	+0.85
24	5.38	3.31	62	4.80	+0.58
25	5.05	2.98	59	3.93	+1.12
26	4.86	3.22	66	3.95	+0.91
27	5.22	4.63	89	4.71	+0.51
28	5.22	4.63	89	4.71	+0.51

TABLE 3.—*Total and Inorganic Iron Content of Various Foods*

Food	Total Iron, Mg. per 100 Gm.	Range in Samples Analyzed	Inorganic Iron, Mg. per 100 Gm.	Range in Samples Analyzed	Number of Lots Analyzed	Inorganic Iron, Percentage
Powdered milk.....	1.30	(1.12-1.65)	1.21	(0.99-1.64)	3	93
Orange juice.....	0.52	(0.31-0.80)	0.52	(0.31-0.80)	28	100
Strained peas.....	1.40	(1.20-1.70)	1.30	(0.84-1.62)	18	93
Strained spinach.....	0.90	(0.72-1.02)	0.50	(0.44-0.56)	6	56
Cream of Wheat.....	0.61	(0.57-0.67)	0.50	(0.50-0.50)	3	82
Special Baby Ralston.....	3.29		3.20		1	97
Beef (ground and cooked)..	2.83	(2.52-3.05)	0.88	(0.70-0.95)	4	31
VioBin.....	58.50		53.80		1	92

closely than the intake of inorganic iron. However, the fact that the retention in periods 7 to 9 was slightly lower than that in periods 1 to 6, whereas the total intake of iron was higher, also indicates that retentions do not always follow the total intake of iron.

A wavelike trend, similar to that found by Porter-Levin²⁰ in her studies of retention of calcium and phosphorus, may be noticed throughout this entire study. If only the total iron is considered, a tendency toward increased retentions with higher intakes is seen. For instance, the average retention in periods 1 to 6, with the total intake of iron at 2.6 mg., was 0.25 mg., whereas the average retention during periods 7 to 12, with the total intake of iron at 3.6 mg. was 0.35 mg.

The amount of hemoglobin fell during the first six periods but tended to show slightly higher values during periods 7 to 12 when the total intake of iron was higher. No differences were noted which could be attributed in any way to the inorganic iron content of the diet. However, since almost 80 per cent of the values of hemoglobin for the entire study lay between 9.7 and 10.7 Gm. per hundred cubic centimeters of blood, it was felt that no significance could be attached to results showing such slight variation.

This lack of significant change in the hemoglobin content of the blood is not surprising when the rapidly changing volume of the blood is considered. During the period of the study the subject gained 4.8 Kg. in weight. For an infant of his age, the gain in weight would correspond to a gain of 370 cc. in the blood volume if MacIntosh's²¹ figure of 77 cc. of blood per kilogram of body weight were used and an even greater gain if the larger figure of 86 cc., as reported by Darrow and his colleagues²² were used. This would represent an increase of 37 Gm. of total circulating hemoglobin in the case of the infant studied, as the average hemoglobin content of his blood was 10 Gm. per hundred cubic centimeters. Since the hemoglobin molecule contains 0.34 per cent of iron, the gain in hemoglobin would mean an increase of 126 mg. in the total amount of circulating iron. The total amount of iron retained during the 28 periods was 56.9 mg., or an average of 0.41 mg. daily. Assuming that the infant stored iron at this average rate for the entire time of the study (two hundred and thirty-eight days), the total storage would have been only 96.6 mg. This is less than the amount needed for the computed increase in hemoglobin, and it is evident that under these conditions a higher concentration of hemoglobin in the blood could not have been expected.

20. Porter-Levin, T.: Calcium and Phosphorus Metabolism of Normal Preschool Children: Successive Balance Studies Showing the Range of Variation in Calcium and Phosphorus Storage, *J. Am. Dietet. A.* **9**:22 (May) 1933.

21. MacIntosh, R.: The Determination of the Circulating Blood Volume in Infants by the Carbon Monoxide Method, *J. Clin. Investigation* **7**:203 (June) 1929.

22. Darrow, D. C.; Soule, H. C., and Buckman, T. E.: Blood Volume in Normal Infants and Children, *J. Clin. Investigation* **5**:243 (Feb.) 1928.

There is no conclusive evidence, then, in either the retentions of the values of hemoglobin found during the first twelve periods, that this child could utilize the iron of peas, which is 93 per cent inorganic iron, better than the iron of spinach, which is only 56 per cent inorganic iron.

It would have been preferable to have a more pronounced difference in the levels of inorganic iron, but this was impossible on account of the fact that in practically all foods suitable for use in the diets of infants 90 to 100 per cent of the iron is in the inorganic form. A large number of strained fruits and vegetables and refined cereals were analyzed, and spinach alone was found to contain appreciable amounts of iron that did not react with the α,α -dipyridine. Results on spinach consistently showed that about 56 per cent of its iron was in the ionizable form. This is a somewhat higher proportion than was found by Elvehjem and his co-workers,²³ but it agrees well with the results of Shackleton and McCance.¹⁶ The difference between results from various laboratories may be due to the different varieties of spinach used, although the fact that fresh material was analyzed in some cases and a cooked, strained product in others would undoubtedly influence the results.

During periods 13 to 20, the availability of iron as an inorganic iron salt, soluble ferric pyrophosphate, was compared with that of the iron in a commercial wheat germ product, the total iron content of which had been shown by analyses to be practically all in the inorganic form. Both the total intake of iron and the intake of inorganic iron were kept as constant as possible throughout these eight periods. The average daily retention was $+0.57$ mg. for periods 13 to 16, with an average total intake of 4.8 mg. of iron, 92 per cent of which was inorganic, as compared to an average daily retention of -0.04 mg. for periods 17 to 20, with an average total intake of 4.8 mg. of iron, 88 per cent of which was inorganic. The values of the hemoglobin during this time tended to remain at a higher level despite the negative balances in periods 18 and 19.

The definite lowering of the retention when the wheat germ product was given suggests the possibility that it was influenced by the increased vitamin B content of the diet. This same effect on the iron balance was noticed by us¹³ when an extract of vitamin B was given to an anemic child. The child, who had had a strongly positive iron balance, stored little iron while the vitamin was added to the diet. There was no noticeable change in the consistency or frequency of the stools in either case. The additional vitamin given to the subject of this study

23. Elvehjem, Hart and Sherman.^{1a} Sherman, Elvehjem and Hart.^{1b} Kohler, Elvehjem and Hart.¹⁷

amounted to only 26 international units²⁴ daily, which is not considered excessive for a child of his age. The fact that low retention occurred during period 16, before the feeding of wheat germ was begun, suggests another possible explanation. It is possible that period 16 marked the beginning of a series of low retentions and that on this level of intake such retentions would have continued, regardless of the type of food given. A further investigation is being planned in regard to the effect on the iron balance of the addition of small amounts of vitamin B to a normal diet.

It will be noted in the chart that the study was interrupted between periods 18 and 19, during the time that the wheat germ product was given. This interruption was due to a slight infection of the upper respiratory tract. The study was discontinued at the onset of the infection and was not resumed until the child had recovered completely. A similar interruption occurred between periods 12 and 13, and since exceptionally high retentions were found in that instance, it does not seem probable that the low retentions in periods 17 to 20 could be related in any way to the infection.

In periods 21 and 22, when the intake was reduced to the initial low level of 2.6 mg. per day, the retention was $+0.20$ mg., closely approximating the retention with a similar intake during periods 1 to 6, when the child was much younger. However, when beef was introduced into the diet, which almost doubled the total intake of iron while it raised the intake of inorganic iron only 45 per cent, the retention of iron was consistently higher than it had been at any previous time on similar intakes. During periods 23 to 26 the average daily retention was $+0.87$ mg., as compared to $+0.57$ mg. during periods 13 to 16. The values of hemoglobin, however, showed no gain during these periods.

In periods 27 and 28, although the total intake of iron was kept at the same level of approximately 5 mg., the inorganic iron constituted 89 per cent of the total, as compared to 62 per cent during the four periods just previous. The average retention was only $+0.51$ mg., and the hemoglobin continued to be low. The retentions shown for the last two periods are in reality those for one period of ten days. It was represented on the chart as two periods because the time involved was double that of any other period. Difficulty was encountered in separating the feces for the two periods, and it was considered best to present average results. During the last few days of period 28 the weather was very warm, and parts of feedings were refused. However, the total amount refused did not appreciably affect the intake, and it would seem that the drop in retention was of significance, since the average

24. Dr. Elizabeth Knott determined the vitamin B content of VioBin by rat assay, using standard technic.

daily retention (+0.51 mg.) was nearly the same as that in periods 13 to 16 (+0.57 mg.), when the child was receiving a similar diet and intake.

The higher retentions which were found when beef was fed certainly show that the child could utilize the iron of beef, of which a low percentage is inorganic. The fact that retentions during periods 23 to 26 were higher than in any of six other periods in which the total intake of iron was approximately the same while the intake of inorganic iron was much higher makes it quite conclusive.

The intake of copper did not vary appreciably in the periods under comparison, as is shown in table 1. In periods 27 to 28 it was higher than in the two groups of periods which preceded them, but the higher level seemed to have no beneficial influence on the hemoglobin.

The intake of iron (0.3 to 0.4 mg. per kilogram) in the early part of the study was low as compared to the requirement of preschool children of 0.5 to 0.6 mg. per kilogram as recommended by Rose and her associates,²⁵ Leichsenring and Flor²⁶ and Daniels and Wright.²⁷ However, Elvehjem and his collaborators^{1c} considered it necessary that subminimal amounts of iron be fed if the availability of the iron is under consideration. Accordingly, in the first twelve periods the intake was as low as seemed advisable when the health of the child was taken into consideration. In the later periods, the intake nearly reached the optimum on the basis of milligram per kilogram requirements for preschool children. While the infant in this study had a positive iron balance at all times, with the exception of two of the periods in which wheat germ was fed, the total retentions as well as the retentions per kilogram increased markedly on the higher intakes. Since the retentions were still increasing when the study came to an end and since the hemoglobin remained practically constant, it is quite evident that the child was receiving no more than his maintenance requirement of iron. Since the intake during the latter part of the study equaled the standard set for preschool children, it is quite probable that the optimum intake of iron for children of this age on a milligram per kilogram basis is higher than that for older children. This could be accounted for by the more rapid change both in weight and in the volume of the blood at the younger age. Needless to say, studies of other subjects, with higher intakes, are necessary before any definite suggestions can be made.

25. Rose, M. S.: Vahlteich, E. M.; Robb, E., and Bloomfield, E. M.: Iron Requirement in Early Childhood, *J. Nutrition* **3**:229 (Nov.) 1930.

26. Leichsenring, J. M., and Flor, I. H.: The Iron Requirement of the Preschool Child, *J. Nutrition* **5**:141 (March) 1932.

27. Daniels, A. L., and Wright, O. E.: Iron and Copper Retentions in Young Children, *J. Nutrition* **8**:125 (Aug.) 1934.

SUMMARY

In this study the availability of inorganic and organic iron from different sources was determined by studying the retention of iron in a normal child.

With a low level of intake no difference was found in the retention when the inorganic iron of the diet was increased, the total intake of iron remaining constant. With a slightly higher level, higher retentions were found in two of three periods when the inorganic iron was increased. However, the total amount of iron was also higher in the periods of increased retention. Therefore, no conclusive evidence was obtained that only the inorganic iron was utilized.

Retentions were found to be much higher with a diet which contained iron and copper salts than with one which contained the same amount of iron and copper in the form of a wheat germ product. The amount of inorganic iron was approximately the same in the two diets, showing that some other factor was present. It is suggested that this factor may be the vitamin B in the wheat germ.

With a diet containing cooked beef which was low in inorganic iron, the retention of iron was consistently higher than with other diets which contained the same amounts of total iron and much larger amounts of inorganic iron. The iron of beef, of which a large portion is organic, was well utilized.

The data, therefore, indicate that in the case of the subject studied the availability of the iron in the food could not be determined by its reaction to α,α -dipyridine.

It is suggested that the optimal requirement of iron for a child less than 1 year of age is more than 0.5 mg. per kilogram.

Helpful criticism was given and suggestions were made by Dr. Lydia J. Roberts of the Department of Home Economics of the University of Chicago.

